Dietary fish oil–induced changes in the distribution of α-tocopherol, retinol, and β-carotene in plasma, red blood cells, and platelets: modulation by vitamin E[1–3]

Padmanabhan P Nair, Joseph T Judd, Elliott Berlin, Philip R Taylor, Samina Shami, Eduardo Sainz, and Hemmige N Bhagavan

ABSTRACT Healthy men (ages 24–57 y) were fed a controlled basal diet supplemented with 15 g/d of placebo oil (PO) for 10 wk followed by 15 g/d of fish-oil concentrate (FO) (fortified with 15 mg all-*rac*-tocopherol) for 10 wk without additional α-tocopherol and the last 8 wk with 200 mg α-tocopherol/d (FO + E). Compared with PO, FO raised plasma malondialdehyde; lowered α-tocopherol in plasma, red blood cells, and platelets; and raised plasma and platelet β-carotene. Supplementation with additional α-tocopherol (FO + E) not only restored tocopherol concentrations but also reversed the rise in β-carotene. The response in retinol, particularly in platelets, showed an inverse relationship to β-carotene, α-tocopherol exhibiting a modulating effect on these changes. From these observations it is postulated that platelets may be a significant extraintestinal site of retinol formation from β-carotene. *Am J Clin Nutr* 1993;58: 98–102.

KEY WORDS n−3 Polyunsaturated fatty acids, α-tocopherol, β-carotene, retinol, fish oil, red blood cells, platelets, vitamin A, vitamin E, polyunsaturated fatty acids

Introduction

Diets rich in seafood or fish oils containing n−3 polyunsaturated fatty acids (PUFAs) such as the long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are considered to be beneficial in the prevention of cardiovascular diseases and cancer (1–6). Suggested modes of action are through their effects on the synthesis of vasoactive prostanoids (7–9), and their hypolipidemic effects (10). Because EPA and DHA are highly unsaturated long-chain fatty acids, they are highly susceptible to lipid peroxidation (11). Under conditions of high dietary intakes of EPA and DHA, the excretion of urinary malondialdehyde (MDA), a principal end product of lipid peroxidation, is elevated (12), probably reflecting an amplification of cellular hydroperoxide production. Thus, ingestion of n−3 PUFAs may contribute to an overall enhancement of prooxidant stress in the body resulting in lower concentrations of endogenous antioxidants, such as vitamin E (13–15), and indirectly affecting other labile micronutrients such as retinol.

To examine in depth several different indexes that are likely to be affected by ingestion of fish oils in humans, we conducted a large multiinvestigator collaborative study at the Human Study Facility of the Beltsville Human Nutrition Research Center in Beltsville, MD (16–18). In this report we present data on the influence of dietary fish oil with and without supplementation with additional vitamin E on the distribution of α-tocopherol, retinol, and β-carotene in some of the major compartments of blood.

Subjects and methods

Subjects

Nonsmoking men between the ages of 24 and 57 y were recruited from the Greater Beltsville, MD, area for an initial interview to determine eligibility for entry into the study. The subjects were screened by means of dietary and medical questionnaires designed to exclude those with health problems such as metabolic disorders, history of any organic disease, regular use of prescription or nonprescription medications, alcohol and dietary habits that are nonrepresentative of the general population.

Those who met the initial selection criteria and gave informed consent were examined by a physician from the Georgetown University School of Medicine. The study protocols were approved by the Institutional Review Boards of the University, the National Cancer Institute, and the US Department of Agriculture (USDA). Part of the medical evaluation before entry consisted of a medical history, hematological profile, blood

[1] From the Lipid Nutrition Laboratory, Beltsville Human Nutrition Research Center, US Department of Agriculture, Agricultural Research Service, Beltsville, MD; the Department of Biochemistry and the Center for Human Nutrition, Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; the Cancer Prevention Studies Branch, Division of Cancer Prevention and Control, National Cancer Institute, National Institutes of Health, Bethesda, MD; and the Department of Clinical Nutrition, Hoffmann-La Roche Inc, Nutley, NJ.

[2] Supported in part by funds from an NCI/USDA Interagency Agreement (YO-1-CN-40620) and by a Grant-in-Aid from Hoffman-La Roche Inc, Nutley, NJ.

[3] Address reprint requests to PP Nair, Lipid Nutrition Laboratory, Beltsville Human Nutrition Research Center, Building 308, USDA, Beltsville, MD 20705.

Received August 14, 1992.

Accepted for publication February 1, 1993.

chemistry evaluation including plasma α-tocopherol concentrations, and urinalysis. Those with body weights < 90% or > 120% of the 1983 standards for desirable weights of the Metropolitan Life Insurance Co were excluded from the study (19). Similarly, those with plasma α-tocopherol concentrations < 13.9 μmol/L or > 27.9 μmol/L were also excluded from the study. Aspirin, aspirin-containing drugs, and other antiinflammatory drugs were not permitted during the study. Occasional use of Tylenol (McNeil-PPC, Inc, Fort Washington, PA) was the only analgesic approved for use when there was an acute need. Antibiotics and other medications taken anytime during the study under the direction of a physician were carefully evaluated for their possible effects on study measurements. From the initial pool of eligible subjects, 41 were selected for participation in the study. Of these, 40 subjects completed the study.

Diets and dietary supplements

A basal diet, planned from commonly available foodstuffs, was designed to contribute a total of 40% of energy from fat when fed in conjunction with 15 g/d of either placebo oil (PO) or fish-oil concentrate (FO) (ROPUFA, 50%; Hoffmann-La Roche Inc, Nutley, NJ). The cholesterol intake was adjusted to $\approx$360 mg $\cdot$ d^{-1} $\cdot$ 11.7 MJ $^{-1}$. The nutrient composition of the diets was calculated by using the USDA *Lipid Nutrition Laboratory Food Database*, derived from data on food composition from the USDA Handbook 8 (20), the food industry, the Nutrient Coding Center in Minneapolis, and by analysis. The contents of the long-chain (20-C, 22-C, and 24-C) n$-$3 fatty acids were minimized in the basal diet by excluding fish from the diet, whereas vitamin E intake was minimized by excluding highly fortified foods from the diet. A 14-d menu cycle ensured variety and acceptability of the meals provided. No vitamins, minerals, or other supplements or alcohol were permitted during the study. The meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC), where subjects came for breakfast and dinner. During weekdays a carryout box lunch was provided whereas weekend meals were packaged and taken home on Fridays. Subjects were initially assigned to an appropriate energy intake based on their estimated need for weight maintenance. Subsequently, body weight was maintained by adjusting the energy intake of the subjects. Consumption of coffee, tea, and water was unrestricted.

After a free-living period (baseline) lasting for 2 wk, all subjects were placed on the controlled basal diet for a total of 28 wk, divided into three periods. During period 1, which lasted 10 wk, the subjects consumed 15 g PO (seven capsules at breakfast and eight capsules at dinner) provided as 1 g soft gelatin capsules fortified with 1.0 mg all-*rac*-α-tocopherol/g oil. During period 2, which lasted for another 10 wk, instead of the PO, the subjects consumed 15 g FO, (each capsule contained 1.0 mg all-*rac*-α-tocopherol/g oil), indistinguishable in appearance from capsules containing PO. The FO capsules were administered in two divided doses of seven capsules at breakfast and eight at dinner. During period 3 the subjects continued to consume FO capsules and received, in addition, a capsule containing 200 mg of all-*rac*-α-tocopherol (Hoffmann-La Roche Inc) at breakfast. Total α-tocopherol intake from the diet and from PO or FO capsules was at least 22 mg/d throughout the study.

The PO was a blend of 48% stripped lard, 40% beef tallow (hormone-free; Canada Packers, Toronto, Canada), and 12% corn oil. The n$-$3 fatty acid supplement was a 50% concentrate of refined anchovy oil (ROPUFA 50%), containing a small amount of retinol (10.2 mg/g). The fatty acid composition of both these supplements was reported earlier (21).

Compliance was high in this study as would be expected because the subjects consumed all of the supplements in the human study facility in the presence of the study staff. Body weight was monitored throughout the study. Bleeding times, determined at frequent intervals, were within normal limits at all times and the subjects tolerated the supplements well.

Biochemical analyses

Fasting blood samples were obtained during the last week of each of the three periods of the study. They were collected into tubes containing EDTA (5 g/L blood) as the anticoagulant and held for 30 min in an ice bath. Plasma, platelets, and red blood cells were separated as follows. The blood was centrifuged at 150 $\times$ *g* for 10 min and the platelet-rich plasma was separated from the red blood cells and recentrifuged at 2000 $\times$ *g* to obtain the platelet pellet and the supernatant plasma. This pellet was resuspended in 3.5 mL cold physiological saline containing 5 g EDTA/L and vortexed. The suspension was centrifuged at 2000 $\times$ *g* for 10 min and the pellet was briefly exposed to hypotonic saline (0.2% saline) to remove any contaminating red blood cells. The platelets were washed two more times with saline and then stored at $-$70 °C until analyzed. An aliquot sample was analyzed for protein content. Packed red blood cells were washed three times with saline and the packed cells were resuspended in an equal volume of saline containing pyrogallol (5 g/L). A hematocrit sample was obtained on this suspension before being placed in storage at $-$70 °C.

Plasma lipid peroxides were determined spectrophotometrically by the procedure of Asakawa and Matsushita (22). Alpha-tocopherol, retinol, and β-carotene were determined by reversed-phase HPLC (23, 24). The limits of detection were 30 pg for retinol, 200 pg for β-carotene, and 750 pg for α-tocopherol.

Statistical analysis

The data were analyzed by paired *t* test and analysis of variance and an α value (two-sided) of 0.05 was considered statistically significant (25). All comparisons were considered and the significant ones were reported with appropriate notations.

Results

The principal characteristics of the study population at entry are shown in **Table 1**. Age and morphometric and biochemical characteristics were within the prescribed limits set by the study.

TABLE 1
Characteristics of the study population at entry*

Age (y)	37.9 ± 5.9
Quetelet's index†	25.7 ± 1.9
Plasma lipids (mmol/L)	
Cholesterol	5.0 ± 0.16
Triglycerides	1.07 ± 0.09
α-Tocopherol	21.6 ± 0.93

* $\bar{x}$ ± SEM; n = 40.
† In kg/m^2.

TABLE 2
Nutrient intake during controlled diets*

Nutrient	Placebo	Fish oil
Fat (% of total energy)	40 ± 0.3	40 ± 0.3
Carbohydrate (% of total energy)	46 ± 0.7	46 ± 0.7
Protein (% of total energy)	16 ± 0.3	16 ± 0.3
Cholesterol (mg)†	360 ± 21	360 ± 21
α-Tocopherol (mg)	22 ± 0.6	22 ± 0.6

* $\bar{x}$ ± SEM; n = 40. Average daily intake for a 14-d menu cycle.
† At 11.7 MJ/d.

The calculated nutrient intake during the controlled PO and FO diet periods were similar (**Table 2**). The estimated daily consumption of fatty acids for the same two periods are presented in **Table 3.** The FO-supplemented diet contributed ≈30% of the total polyunsaturates as n−3 PUFAs compared with ≈7% in the PO period.

Table 4 presents data on plasma malondialdehyde during the three periods of the study. Malondialdehyde concentrations rose from 1.65 μmol/L during the PO period to 3.72 μmol/L during FO supplementation. Supplementation with α-tocopherol not only counteracted this rise but reduced it to concentrations below that of the PO period.

Table 5 presents data on the distribution of α-tocopherol in plasma, platelets, and red blood cells. In all three, there was a significant reduction in α-tocopherol concentrations on ingestion of FO. This was effectively counteracted when additional α-tocopherol was administered together with FO in period 3 of the study.

Table 6 shows the distribution of retinol in plasma, platelets, and red blood cells. There was a significant rise in plasma retinol, although within normal limits, during the FO period. Alpha-tocopherol supplementation in period 3 had no further effect on plasma retinol. Platelet retinol concentration, which tended to be slightly depressed during FO feeding, showed a dramatic rise when α-tocopherol was given concurrently with FO. In red blood cells only 32 of the 41 subjects showed any detectable amounts of retinol during the PO period. With FO supplementation followed by FO plus α-tocopherol, the number of subjects showing any detectable retinol in red blood cells steadily declined to 15 in period 2 and then down to 3 in period 3. At a detection limit of 30 pg, the results showed that red blood cells contain barely detectable amounts of retinol.

Table 7 summarizes the data on the distribution of β-carotene in the three blood compartments. In plasma, β-carotene values were significantly higher during the FO period than during the PO period. However, when both FO and vitamin E were given together, β-carotene concentrations tended to be lower than when FO was given alone. The platelets consistently reflected the changes in plasma. The red blood cells had no detectable amounts of β-carotene.

TABLE 3
Fatty acid intake on controlled diets*

Fatty acids	Placebo	Fish oil
	g/d	
Saturated fatty acids	33 ± 1.1	29 ± 1.1
Palmitic (16:0)	20 ± 0.6	18 ± 0.6
Stearic (18:0)	9 ± 0.3	7 ± 0.3
Monoenoic fatty acids	46 ± 0.6	42 ± 0.6
Oleic acid (18:1n−9)	44 ± 0.6	39 ± 0.6
Total polyenoic fatty acids	27 ± 0.6	33 ± 0.6
n−3 Polyenoic fatty acids	2 ± 0.1	10 ± 0.1
Linolenic (18:3n−3)	2 ± 0.1	2 ± 0.1
Eicosapentaenoic (20:5n−3)	0	5 ± 0.01
Docosahexaenoic (22:6n−3)	0	2 ± 0.01
n−6 Polyenoic fatty acids	25 ± 0.6	23 ± 0.6
Linoleic acid (18:2n−6)	25 ± 0.6	23 ± 0.6

* $\bar{x}$ ± SEM at 11.7 MJ/d; n = 40.

TABLE 4
Plasma malondialdehyde*

Phase of study	Malondialdehyde concentration
	μmol/L
Placebo (n = 40)	1.65 ± 0.16
Fish oil (n = 41)	3.72 ± 0.25†
Fish oil + vitamin E (n = 40)	0.98 ± 0.06†‡

* $\bar{x}$ ± SEM.
† Significantly different from placebo, P = 0.0001 (paired t test).
‡ Significantly different from fish oil, P = 0.0001 (paired t test).

Discussion

Long-chain PUFAs of both the n−3 and n−6 series are highly susceptible to lipid peroxidation. As a result, a diet rich in these fatty acids but without an adequate supply of antioxidants could be expected to exert peroxidative stress in vivo. The observations in this study showing a rise in lipid peroxides in plasma after supplementation with FO are consistent with this assumption. Peroxidative stress could lead to an increased turnover of the body reserves of endogenous antioxidants such as vitamin E. The decrease in circulating plasma α-tocopherol could therefore be a reflection of the increased demand for antioxidants. Concurrent supplementation with additional vitamin E overcomes this effect, as seen by the restored concentrations of plasma α-tocopherol. Paradoxically, in studies on the fluidity and composition of erythrocyte membranes we observed a significant rise in both α- and γ-tocopherol concentrations in the red blood

TABLE 5
Alpha-tocopherol in plasma, platelets, and red blood cells*

Phase of study	Plasma	Platelets	Red blood cells
	μmol/L	*μmol/g protein*	*μmol/L packed cells*
Placebo	23.7 ± 0.70†	0.70 ± 0.05†	4.88 ± 0.14†
Fish oil	20.0 ± 0.70	0.46 ± 0.05	2.74 ± 0.07
Fish oil + vitamin E	26.5 ± 0.93†	0.60 ± 0.02‡	8.22 ± 0.39†

* $\bar{x}$ ± SEM; n = 40.
†‡ Significantly different from fish oil (paired t test): †$P \leq 0.0001$, ‡$P \leq 0.01$.

cell membrane after feeding FO (21). In a recent study on a small group of men on self-selected diets, plasma tocopherol concentrations were reported to have risen after 6 wk on 18 g fish oil/d (26). The authors have attributed this to the vitamin E content (1.35 mg/g) of the supplement, which amounts to ≈24 mg of additional vitamin E. Because these subjects were not on a controlled diet, it is hard to explain these findings. Our data from this study are consistent with the findings from a recent study in rats in which enrichment of the diet with PUFAs resulted in a significant reduction in plasma α-tocopherol (14). The concentration of α-tocopherol in platelets and red blood cells is a reflection of changes observed in plasma.

The distribution of retinol in plasma and platelets did not follow the pattern of α-tocopherol. n−3 PUFA ingestion resulted in a small but significant rise in plasma retinol, which was well within the normal range whereas retinol in platelets tended to be slightly reduced. Supplementation with additional vitamin E caused a marked rise in platelet retinol whereas plasma values were unaffected. The significance of this selective response of platelet retinol to vitamin E is unclear. Furthermore, there seems to be a reciprocal relationship between platelet retinol and β-carotene after FO and vitamin E supplementation. In contrast, red blood cells showed barely detectable concentrations of retinol and no β-carotene. This suggests that retinol and β-carotene may not be significant constituents of red blood cells.

The sharp rise in plasma β-carotene in response to dietary FO may be a reflection of the effect of PUFAs on the absorption of β-carotene. In a recent study in rats, it was shown that animals fed a corn oil–rich diet had higher liver and plasma β-carotene when compared with control animals fed a diet high in saturated fatty acids (27). In our study it is not clear why the concomitant administration of additional α-tocopherol tended to counteract this rise of β-carotene in plasma. It appears that n−3 fatty acids and α-tocopherol have opposite effects on the metabolic regulation of the absorption and/or conversion of β-carotene to retinol. The reciprocal relationship between β-carotene and retinol in platelets also appears to be modulated by α-tocopherol status. This is of particular significance because we recently observed the presence of the β-carotene cleavage enzyme in platelets (MR Lakshman, M Somanchi, HN Bhagavan, PP Nair, unpublished observations, 1992). We speculate that tocopherol is mechanistically involved in the conversion of β-carotene to retinol in platelets either directly or indirectly as a lipid peroxide scavenger. Furthermore, platelets may be a significant site for the extraintestinal conversion of β-carotene to retinol.

TABLE 6
Retinol in plasma, platelets, and red blood cells*

Phase of study	Plasma	Platelets	Red blood cells†
	μmol/L	*nmol/g protein*	*nmol/L packed cells*
Placebo	1.72 ± 0.05 [41]‡	1.09 ± 0.17 [41]	20.1 ± 1.75 [41]‡
Fish oil	2.02 ± 0.07 [41]	0.73 ± 0.14 [41]	6.77 ± 1.43 [41]
Fish oil + vitamin E	1.99 ± 0.07 [40]	2.83 ± 0.24 [40]†	1.36 ± 0.77 [40]†

* $\bar{x}$ ± SEM; *n* in brackets.
† Subjects showing detectable amounts of retinol: placebo (n = 32), fish oil (n = 15), fish oil + vitamin E (n = 3); in subjects with no detectable retinol, 0 was used.
‡ Significantly different from fish oil, $P \leq 0.0001$ (paired t test).

TABLE 7
β-Carotene in plasma, platelets, and red blood cells*

Phase of study	Plasma	Platelets	Red blood cells
	μmol/L	*nmol/g protein*	*nmol/L packed cells*
Placebo	0.36 ± 0.02 [41]†	6.59 ± 0.78 [41]‡	ND
Fish oil	0.52 ± 0.03 [41]	11.3 ± 1.49 [41]	ND
Fish oil + vitamin E	0.40 ± 0.02 [40]	6.11 ± 0.52 [40]†	ND

* $\bar{x}$ ± SEM; *n* in brackets. ND, not detected.
†‡ Significantly different from fish oil (paired t test): †$P < 0.0001$, ‡$P < 0.0025$.

Another important observation in this study is the relatively low concentrations of retinol and the absence of β-carotene in red blood cells. This is consistent with the data from a previous study showing the absence of detectable amounts of both retinol and β-carotene in red blood cells in human subjects (28). Both n−3 fatty acids and vitamin E may be involved in the regulation of retinol and β-carotene concentrations in plasma and platelets. These compounds are apparently not necessary for red blood cell function and furthermore red blood cells may lack the binding proteins for retinol and β-carotene. Additional studies are required to ascertain whether lipid peroxidation is mechanistically involved in this process. Furthermore, it would also be important to examine whether a similar regulation exists in other tissues.

References

1. Lands WEM. Fish and human health. New York: Academic Press Inc, 1986.
2. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 1979;2:433–5.
3. Knapp HR. Polyunsaturated fatty acids and cardiovascular function in man. Klin Wochenschr 1990;68:33–9.
4. Neilsen NH, Hansen JPH. Breast cancer in Greenland: selected epidemiological, clinical and histological features. J Cancer Res Clin Oncol 1980;98:287–99.
5. Blot WJ, Lanier A, Fraumeni JF Jr, Bender TR. Cancer mortality among Alaskan natives 1960–1969. J Natl Cancer Inst 1975;55: 547–54.
6. Karmali RA, Marsh J, Fuchs C. Effects of omega-3 fatty acids on growth of a rat mammary tumor. J Natl Cancer Inst 1984;73:457–61.
7. Dyerberg J, Bang HO, Stofferson E, Moncada S, Vane JR. Eicosapentenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1978;2:117–9.
8. Leaf A, Weber PC. Cardiovascular effects of n−3 fatty acids. N Engl J Med 1988;318:549–57.
9. Knapp HR, FitzGerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Engl J Med 1989;320:1037–43.
10. Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res 1989;30:785–807.
11. Yagi K. Lipid peroxidation and human diseases. Chem Phys Lipids 1987;45:337–51.
12. Wahle KWJ, Brown JE. Plasma lipid peroxidation and vitamin E supplementation during high intakes of fish oil fatty acids. Fat Sci Technol 1990;92:326–30.
13. Meydani SN, Shapiro AC, Meydani MM, Macauley JB, Blumberg JB. Effect of age and dietary fat (fish, corn and coconut oils) on tocopherol status of C57BL/6Nia mice. Lipids 1987;22:345–50.

14. Chautan M, Calaf R, Leonardi J, et al. Inverse modification of heart and liver α-tocopherol status by various dietary n−6/n−3 polyunsaturated fatty acid ratios. J Lipid Res 1990;31:2201–8.
15. Harmon EM, Witting LA, Horwitt MK. Relative rates of depletion of alpha-tocopherol and linoleic acid after feeding polyunsaturated fats. Am J Clin Nutr 1966;18:243–7.
16. Ferretti A, Judd JT, Ballard-Barbash R, Nair PP, Taylor PR, Clevidence BA. Effect of fish oil supplementation on the excretion of the major metabolite of prostaglandin E in healthy male subjects. Lipids 1991;26:500–3.
17. Bhathena SJ, Berlin E, Judd JT, et al. Effects of ω3 fatty acids and vitamin E on hormones involved in carbohydrate and lipid metabolism in men. Am J Clin Nutr 1991;54:684–8.
18. Kramer TR, Schoene N, Douglass LW, et al. Increased vitamin E intake restores fish-oil-induced suppressed blastogenesis of mitogen-stimulated T lymphocytes. Am J Clin Nutr 1991;54:896–902.
19. Metropolitan Life Insurance Company. 1983 Height and weight tables. Stat Bull Metropol Insur Co 1984;64:2–9.
20. US Department of Agriculture. Composition of foods. Agriculture handbook no. 8. Washington, DC: US Government Printing Office, 1976–1988.
21. Berlin E, Bhathena SJ, Judd JT, et al. Effects of omega-3 fatty acid and vitamin E supplementation on erythrocyte membrane fluidity, tocopherols, insulin binding, and lipid composition in adult men. J Nutr Biochem 1992;3:392–400.
22. Asakawa T, Matsushita S. Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids 1980;15:137–40.
23. Stacewicz-Sapuntakis M, Bowen PE, Kikendall JW, Burgess M. Simultaneous determination of serum retinol and various carotenoids: their distribution in middle-aged men and women. J Micronutr Anal 1987;3:27–45.
24. Craft NE, Brown ED, Smith JC. Effects of storage and handling conditions on concentrations of individual carotenoids, retinol and tocopherol in plasma. Clin Chem 1988;34:44–8.
25. Snedecor GW, Cochran WF. Statistical methods. 7th ed. Ames, IA: The Iowa State University Press, 1980.
26. Shapiro AC, Meydani SM, Meydani M, et al. The effect of fish oil supplementation on plasma α-tocopherol, retinol, lipid and lipoprotein levels in normolipidemic subjects. Nutr Res 1991;11:539–48.
27. Blakely SR, Dodge WR, Grundel E, Mitchell GV, Jenkins MY. Enhanced accumulation of β-carotene and canthaxanthin in rat liver and plasma with moderately high corn oil diets. Proc Soc Exp Biol Med 1992;200:277 (abstr).
28. Shah SN, Johnson RC, Singh VN. Antioxidant vitamin (A and E) status of Down's syndrome subjects. Nutr Res 1989;9:709–15.